TECHNICAL MANUAL

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INSPIRED CELL SOLUTIONS

Dharmacon™ ORFeome Collaboration Clones and Collections

Cat #OHS4559, OHS4560, OMM4760, OMM4761, OHS5893, OHS5894, OMM5895, OMM5896, OXL11194

Product Description

The Gateway™ cloning system was adopted by the ORFeome Collaboration (OC) for use with all of its clones. This site-specific recombinational cloning system allows for efficient transfer of ORF sequence from one vector (Entry vector) to any other expression vector modified with the requisite recombination sites flanking the insertion site for the ORF (Destination vector). ORFs transferred in this way have been found to acquire sequence changes only very rarely; thus for most purposes transferred sequences require no additional sequence analysis.

The bulk of the OC targets have been generated by Dana Farber Cancer Institute-Center for Cancer Systems Biology, from their program to develop an extensive collection of full-ORF clones for human proteins. Dana Farber has transferred into Gateway™ Entry vectors the ORF sequences from a majority of the existing MGC full-ORF human cDNA clones. Full-length sequence validation of these clones is then conducted at Welcome Trust Sanger Institute.

More information is located on the following website:
orfeomecollaboration.org
xenbase.org/reagents/static/orfeome.jsp

Clones are provided as bacterial cultures of DH10B TonA *E.coli* in LB medium with 8% glycerol, and the appropriate antibiotic as indicated in the table below.

Table 1.

Cap Color	Antibiotic	Concentration
green	Kanamycin	25 µg/mL
brown	Spectinomycin	100 µg/mL

Shipping And Storage

Individual clones are shipped at room temperature and may be stored for up to one week at +4 °C. They may stored indefinitely at –80 °C. Plates are shipped on dry ice and should be stored at –80 °C. All cultures are checked for growth prior to shipment.

To allow any CO_2 that may have dissolved into the medium from the dry ice in shipping to dissipate, please store plates at –80 °C for at least 48 hours before thawing.

Obtaining Clone Information

The Dharmacon Search provides a rapid means of obtaining clone information. Enter the Clone ID or catalog number of your clone in the query box. Expand the clone information by clicking on the "+" symbol on the left of the clone description. The vector information is in the "Details" tab and the sequence accession number is proviced in the "Sequence" tab. The accession link will take you to NCBI for the sequence of the clone of interest (See Figure 1).

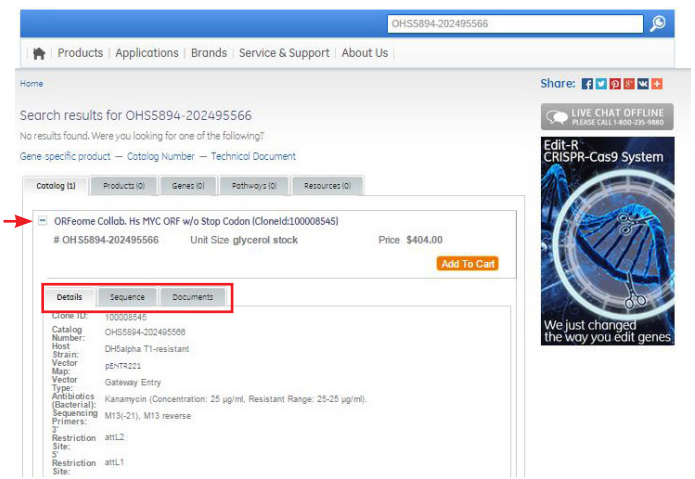

Figure 1. Dharmacon Search

Cloning Method Overview

Wellcome Trust Sanger Institute

ORFs flanked by att sites were amplified from fully sequence verified cDNA clones in two rounds of PCR. Amplified products were separated by agarose gel electrophoresis, excised, purified and recombined into a Gateway™ vector. After full sequence verification, only clones which exactly matched the original cDNA and att sequences were accepted.

Dana Farber Cancer Institute

MGC clones were cherry-picked into a non-redundant set and 8 ul of each clone was inoculated in 1 mL LB containing either ampicillin (100 µg/mL) or chloramphenicol (34 µg/mL) depending on the MGC vector. A BP recombinational reaction contains 2 µL of 5 × BP3 buffer; 2 µL of BP clonase; 1 µL of pDONR223 (150 ng/µL); 2 µL of PCR product (2-200 ng/µL); 3 µL H_2O. The 5 × BP3 buffer consists of 100 mM Tris-Cl (pH 7.5); 20 mM EDTA; 30 mM spermidine-HCL; 25% glycerol; 225 mM NaCl. LR reactions we performed as described previously with minor changes (Reboul *et al*. 2003, Rual *et al*. 2004). BP products were transformed into liquid cultures of *E. coli,* with antibiotic selection of spectinomycin at 50 µg/mL.

Clone Replication Protocol

Once the clone has been streak isolated and the identity of the strain has been confirmed as prescribed in Protocol I below we recommend making a stock of the pure culture. Grow the pure culture in LB broth + appropriate antibiotic. Transfer 920 µL of culture into a polypropylene tube and add 80 µL sterile glycerol to make an 8% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at -80 °C.

Protocol I-Verifying Individual Clone Identity

We recommend picking at least 5 independent colonies for verification to ensure that the clone of interest is derived from a single isolate either by sequencing or restriction digestion.

By Sequencing

We further recommend verification of clones by end sequencing. The sequencing primers appropriate for each vector can be obtained from the clone query by clicking on the accession number of the clone result. A useful tool for comparing the sequence obtained to the sequence expected is to perform a pairwise BLAST. The link to this feature on the NCBI website at: blast.ncbi.nlm.nih.gov/Blast.cgi. Simply enter the sequence you obtained in the Sequence 1 window and enter the sequence retrieved from the Clone Details screen in the Sequence 2 window (Figure 2).

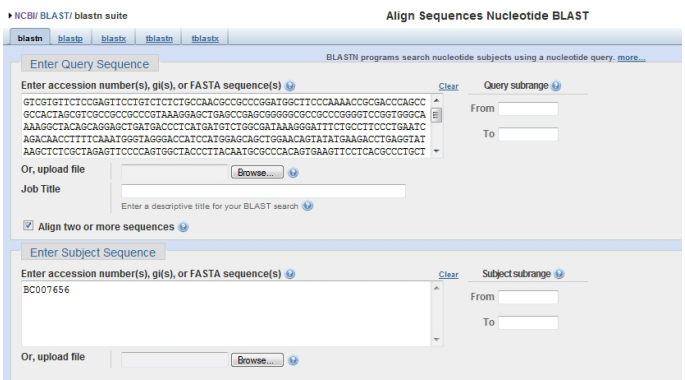

Figure 2. Pairwise BLAST (webshot courtesy of the NCIB).

By Restriction Digestion

To locate the restriction enzymes used to construct a particular clone, use the clone query and clicking on the accession number of the clone result. This section contains all available information about how each cDNA was cloned.

- **A helpful restriction mapping tool is located at restrictionmapper.org.**
- **Vector maps and sequences for some vectors may be downloaded from our website.**

Protocol II-Plate Replication

Table 2. Materials

Item	Vendor	Cat #
LB-Lennox Broth (low salt)	VWR	EM1.00547.0500
Glycerol	VWR	EM-4760
Spectinamycin	Calbiochem	567570
Kanamycin	VWR	80058-286
96-well microplates	VWR	62407-174
Aluminum seals	VWR	73520-056
Disposable replicators	Genetix	X5054

Plate Replication Protocol

Prepare Target Plates

- Dispense ~160 µL of sterile LB media into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare Source Plates

- Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
- Thaw the source plates with the lids on. Wipe any condensation underneath the lid with a Kimwipe dampened with alcohol.

Replicate

- Gently place a disposable replicator into the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the plate of the well.
- Carefully remove the replicator from the source plate and gently place the replicator into the target plate. Gently move the replicator back and forth in the target plate to transfer cells.
- Discard the replicator.
- Place the lids back on the source plates and target plates.
- Seal the source plates, being mindful to avoid cross contamination.
- Repeat this process until all plates have been replicated.
- Return the source plates to the –80 ºC freezer.
- Place the inoculated target plates in a 37 ºC incubator. Incubate the plates for 12–24 hours.

If Your Clone Is Not Growing

For individual clones, we use an inert growth indicator to check each culture for growth prior to shipping. If your clone is growing slowly or not growing for you, try the following suggestions:

- Try using broth culture instead of plate culture to jumpstart growth.
- Use shaking during growth of the broth culture.
- Ensure that you are using the correct antibiotic for your ORF.
- Try inoculating from thawed source tube rather than frozen.
- Gently mix the source tube by inversion (with the lid on) to ensure the cells are not settled in the bottom before the inoculum is taken.
- Spin the tube down and streak directly from the pellet

References

1. Hartley, J.L., Temple, G.F. and Brasch, M.A. (2000) DNA cloning using in vitro site-specific recombination. *Genome Res*, **10**, 1788-95.
2. Invitrogen (2006) invitrogen.com
3. Rual, J.F., Hirozane-Kishikawa, T., Hao, T., Bertin, N., Li, S., Dricot, A., Li, N., Rosenberg, J., Lamesch, P., Vidalain, P.O. *et al.* (2004) Human ORFeome version 1.1: a platform for reverse proteomics. *Genome Res*, **14**, 2128-35.
4. Lamesch, P., Li, N, Milstein, S *et al.* (2006) hORFeome v3.1: *A Resource of human open reading frames covering over 10,000 Human Genes.*
5. Reboul J,et al. C.elegans ORFeome version 1.1 experimental verification of the genome annotation and resource for proteome-scale protein expression. *Nature Genetics volume*, **34**, May 2003
6. orfeomecollaboration.org

FAQS/Troubleshooting

For answers to questions that are not addressed here, please email technical support at ts.dharmacon@horizondiscovery.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

Licensing Information

AGREEMENT IN GOOD FAITH CONCERNING USE AND DISTRIBUTION OF ARRAYED ORFEOME COLLABORATION cDNA CLONES

You are being provided with ORFeome Collaboration [OC] cDNA clones (CLONES) and/or associated products (PRODUCTS) (referred to collectively as OC MATERIALS), in order to advance the public interest and to advance the objectives of the institutions that developed the original libraries from which these clones were derived (ORIGINATORS). The ORIGINATORS are the beneficiaries of, and may independently enforce, this Agreement.

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ADMINISTRATION

Any correspondence concerning this Agreement should be addressed to: Lawrence Livermore National Laboratory. The Regents of the University of California Industrial Partnerships and Commercialization Program Attn: IMAGE Consortium P.O. Box 808, L-795 Livermore, CA 94550 Phone: (925) 422-6416 Fax: (925) 423-8988 llnl.gov

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